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The synergistic effects of lysophosphatidic acid receptor agonists and calcitriol on MG63 osteoblast maturation at titanium and hydroxyapatite surfaces

Jason P. Mansell [a,*], Michele Barbour [a], Christopher Moore [a], Maryam Nowghani [a], Moreica Pabbruwe [b], Terje Sjostrom [a], Ashley W. Blom [b]

[a] Department of Oral & Dental Science, University of Bristol Dental School, Lower Maudlin St., Bristol BS1 2LY, UK
[b] Bristol Implant Research Centre, Department of Clinical Sciences North Bristol, University of Bristol, Southmead Hospital, Bristol BS10 5NB, UK

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ABSTRACT

Successful osseointegration stems from the provision of a mechanically competent mineralised matrix at the implant site. Mature osteoblasts are the cells responsible for achieving this and a key factor for ensuring healthy bone tissue is associated with prosthetic materials will be 1α,25 dihydroxy vitamin D3 (calcitriol). However it is known that calcitriol *per se* does not promote osteoblast maturation, rather the osteoblasts need to be in receipt of calcitriol in combination with selected growth factors in order to undergo a robust maturation response. Herein we report how agonists of the lysophosphatidic acid (LPA) receptor, LPA and (2S)-OMPT, synergistically co-operate with calcitriol to secure osteoblast maturation for cells grown upon two widely used bone biomaterials, titanium and hydroxyapatite. Efforts could now be focussed on functionalising these materials with LPA receptor agonists to support *in vivo* calcitriol-induced osseointegration via heightened osteoblast maturation responses.

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1. Introduction

Escalating costs of revision surgery for total joint replacements of the hip and knee are a stark reminder that current research must extend to ensuring longer-term osseointegration and prosthesis durability. Total hip and knee replacements are effective treatments against osteoarthritic and osteoporotic joint deterioration [1–3]. The former is accepted as a successful surgical procedure that can reduce pain and improve mobility with a failure rate of approximately 10% ten years post implantation [4,5]. However, aseptic loosening is the largest cause of orthopaedic implant failure due to poor initial osseointegration at the prosthesis–host tissue interface [6]. Sound and rapid implant osseointegration is absolutely essential in securing surgical success, reducing revision surgery and resultant patient morbidity and health care costs [7]. It is generally accepted that enhancing osseointegration is dependent upon many variables including implant-related factors, for example the chemical composition and topography of the prosthetic surface [8]. Similarly researchers have looked towards bone fide osteogenic agents to accelerate the host bone growth response at the implant site, e.g., the application of growth factors including bone morphogenetic proteins [9–14] and transforming growth factor beta [15]. However their large size, which will likely compromise their adsorption and/or release from implants together with their high cost are likely to preclude their application. Nevertheless it is entirely pertinent to search for suitable osteogenic factors that have potential in generating superior implant strategies.

The identification of novel agent combinations for promoting human osteoblast maturation is part of our ongoing programme of bone tissue repair and regeneration. A major factor responsible for providing a mechanically competent, mature mineralised bone matrix by osteoblasts is 1α,25 dihydroxyvitamin D_3 [16,17]. Indeed deficiencies of this steroid result in childhood rickets and osteomalacia in adults [18] whereby there is a paucity of adequately mineralised bone matrix. Given the necessity of 1α,25 dihydroxyvitamin D_3 (D3) for healthy bone formation by osteoblasts we have actively sought small biological agents that can act in concert with D3 in driving human osteoblast maturation. We recently discovered that a major serum borne lipid, lysophosphatidic acid (LPA), co-operates synergistically with D3 in promoting human osteoblast maturation [19,20]. In isolation both D3 and LPA elicited only a weak differentiation response in the immature MG63 cell line, however when used in combination the cells responded by increasing the synthesis of both osteoclacin and alkaline phosphatase, proteins expressed by mature osteoblast cells.

To realise the future potential of LPA receptor agonist-D3 pairings in orthopaedic implantology it is essential that an investigation of their ability to promote human osteoblast maturation upon

* Corresponding author. Tel.: +44 117 342 4779; fax: +44 117 342 4428.
E-mail address: j.p.mansell@bris.ac.uk (J.P. Mansell).

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Table 1
Profilometry data for titanium (Ti) and hydroxyapatite (HA)-coated Ti discs. A random batch of ten Ti discs and ten HA-coated Ti discs were processed for profilometry. With regard the former, measurements were recorded after initial cleaning and polishing. No further modifications were made to the HA-coated Ti specimens and these were processed for profilometry as received direct from the manufacturer (DePuys). The area scanned for all samples was 1×1 mm and an assessment of the mean deviation from the flat surface (Ra), the mean peak-trough distance (Rz) and the maximum peak-trough distance (Rmax) quantified. All data are expressed in microns (mean) $\pm$ the standard deviation.

Biomaterial	Ra	Rz	Rmax
Titanium	0.27 ± 0.07	1.63 ± 0.46	1.96 ± 0.51
HA-coated titanium	6.66 ± 0.71	22.02 ± 2.03	30.65 ± 3.18

biomaterials be explored. To this end we grew MG63 cells upon two widely used biomaterials, titanium and hydroxyapatite and assessed their maturation to co-treatment with D3 and either LPA or the LPA 1/3 selective receptor agonist, (2S)-1-oleoyl-2-O-methyl-glycero-3-phosphothionate [21,22].

2. Materials & methods

2.1. Biomaterials preparation and characterisation – profilometry & scanning electron microscopy (SEM)

Orthopaedic grade titanium (Ti) discs and hydroxyapatite (HA) coated Ti discs (both 12.7 mm diameter and 2.5 mm thickness) were generously provided by DePuys CMW. Pure Ti discs were initially polished (Struers TegraPol-15) using silicon carbide paper (grit 500, Struers). A random batch of 10 Ti and 10 HA-coated Ti samples were subsequently processed for profilometry using a Proscan 2000 (Scantron, Taunton, UK) operated from a PC using Proscan software. The area scanned for all samples was 1×1 mm and an assessment of the mean deviation from the flat surface (Ra), the mean peak-trough distance (Rz) and the maximum peak-trough distance (Rmax) quantified. With respect to SEM, samples of tissue culture plastic, Ti and HA-coated Ti

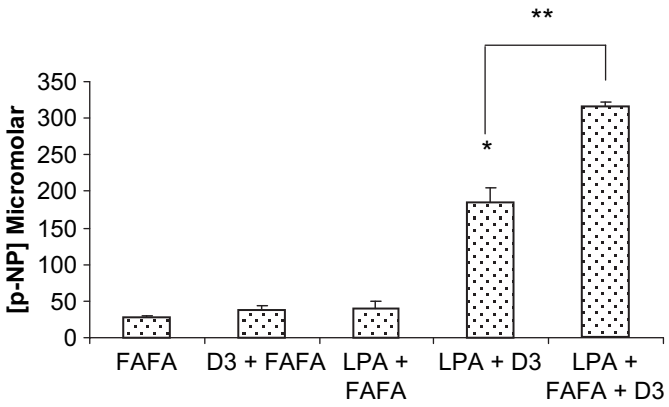

Fig. 2. Fatty acid free albumin (FAFA) augments the maturation response of MG63 osteoblasts to lysophosphatidic acid (LPA) and calcitriol (D3). Osteoblasts were seeded into 24-well culture plates at a density of 2×10^4 cells/mL/well and left for three days prior to an overnight starve in serum free culture medium. Once starved the cells were stimulated with 20 μM LPA and 100 nM D3 in the presence or absence of 250 μg/mL human FAFA. As expected the co-stimulation of MG63 cells with D3 and LPA led to the large synergistic increase ($^*p < 0.001$) in p-nitrophenol (p-NP) from p-NPphosphate reflecting greater alkaline phosphatase activity and therefore cell maturation. This maturation response was heightened further when the cells were stimulated in the presence of FAFA. Indeed the additional increase was a substantial 70% over the LPA and D3 co-treated group ($^{**}p < 0.001$).

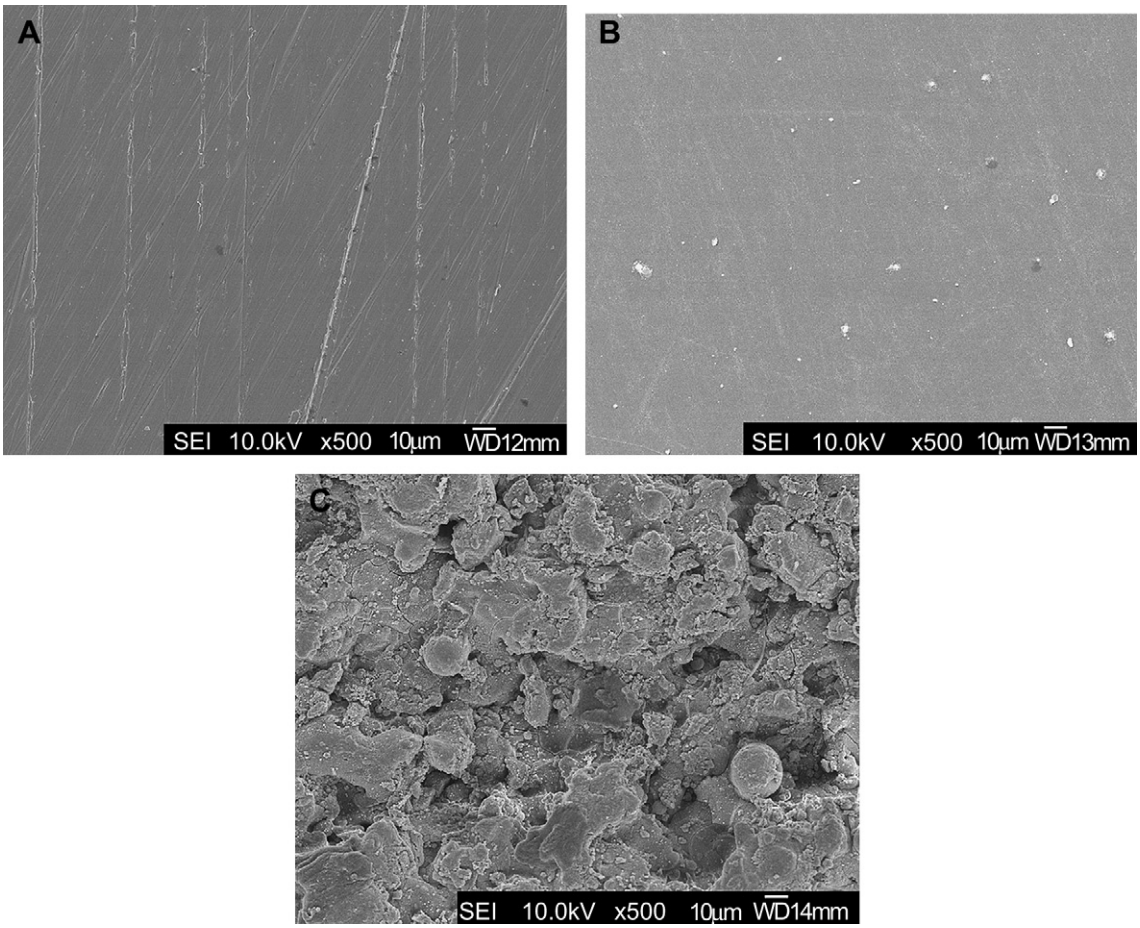

Fig. 1. Scanning electron micrographs of biomaterials. A. Titanium (Ti), B. Tissue culture plastic and C. Hydroxyapatite-coated Ti. For SEM analysis samples were coated with a 15 nm layer of Pt/Pd prior to analysis using an accelerating voltage of 10 kV. Images were taken with a JEOL 6330 FEG-SEM.

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